

Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DN 98-039B

TDR

In re application of: Carlson, et. al.

Serial No.: Not Yet Assigned : Group Art Unit: Not Yet Assigned

Filed: Herewith : Examiner: Not Yet Assigned

For: Ligands for Modulating the Expression of Exogenous Genes Via  
an Ecdysone Receptor Complex

Assistant Commissioner for Patents  
Washington, DC 20231

Dear Sir:

**PRELIMINARY AMENDMENT**

In the above-identified patent application, please enter the following  
amendments:

**In the Specification:**

Page 1, insert the following new paragraph prior to the first paragraph:

This application is a Divisional Application of Application Serial No.  
09/315,451, filed May 20, 1999 which was a Continuation in Part of  
Application Serial No. 09/210,010, filed December 11, 1998, now abandoned,  
which claims the benefit of U.S. Provisional Application No. 60/089,546, filed  
June 17, 1998.

Page 9, the first paragraph is amended to read:

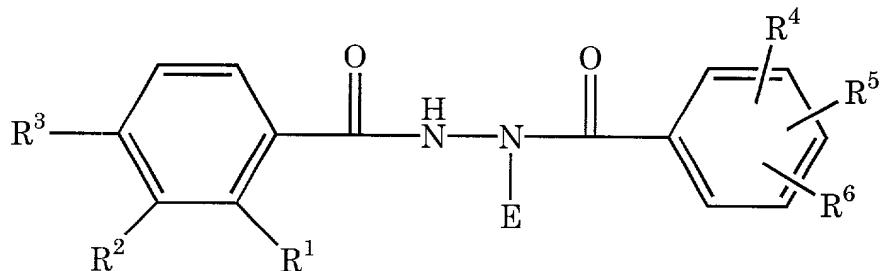
The term "response element" ("RE") means one or more cis-acting DNA  
elements which confer responsiveness on a promoter mediated through  
interaction with the DNA-binding domains of the ecdysone receptor complex.  
This DNA element may be either palindromic (perfect or imperfect) in its  
sequence or composed of sequence motifs or half sites separated by a variable

number of nucleotides. The half sites can be similar or identical and arranged as either direct or inverted repeats. The ecdysone receptor complex binds, in the presence or absence of a ligand, to the DNA sequence of an RE to initiate or suppress transcription of downstream gene(s) under the regulation of this response element. Examples of DNA sequences for RE of the natural ecdysone receptor are known in the art (Cherbas L., et al., (1991), *Genes Dev.*, 5, 120-131; D'Avino PP., et al., (1995), *Mol. Cell. Endocrinol.*, 113, 1-9; Antoniewski C., et al., (1994), *Mol. Cell. Biol.*, 14, 4465-4474).

In the Claims:

Cancel Claim 18.

3. (Amended) A method to modulate the expression of one or more exogenous genes in a subject, wherein the subject is other than a plant, comprising administering to the subject an effective amount of a ligand of the formula:



wherein:

E is a (C<sub>4</sub>-C<sub>6</sub>)alkyl containing a tertiary carbon or a cyano(C<sub>3</sub>-C<sub>5</sub>)alkyl containing a tertiary carbon;

R<sup>1</sup> is H, Me, Et, i-Pr, F, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C<sup>o</sup>CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>;

R<sup>2</sup> is H, Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C<sup>o</sup>CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe<sub>2</sub>, NEt<sub>2</sub>, SMe, SET, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H, COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-i-Pr, SCN, SCHF<sub>2</sub>, SOMe, NH-CN, or joined with R<sup>3</sup> and the phenyl

carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon; R<sup>3</sup> is H, Et, or joined with R<sup>2</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> are independently H, Me, Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SET;

provided that:

a) when R<sup>1</sup> is Me and R<sup>2</sup> is OMe;

then R<sup>3</sup> is H; and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me, 3,5-di-OMe-4-Me, 3,5-di-Cl, or 3,5-di-F;

b) when R<sup>1</sup> is Me and R<sup>2</sup> is OEt;

then R<sup>3</sup> is H and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me, 3,5-di-OMe-4-Me, 3,5-di-Cl, 3,5-di-F, 2,4- or 2,5-di-F, 2,4- or 2,5-di-Cl;

c) when R<sup>1</sup> is Et and R<sup>2</sup> is OMe or OEt;

then R<sup>3</sup> is H and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is:

i) 3,5-di-OMe-4-Me, 3,5-di-Cl, 3,5-di-F, 2,4- or 2,5-di-F, 2,4- or 2,5-di-Cl, 3-OMe, 2-Cl-5-Me, 2-Br-5-Me, 2-Cl, 2-Br, or 3-Me; or

ii) R<sup>6</sup> is H, R<sup>4</sup> is Me, and R<sup>5</sup> is Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SET;

d) when R<sup>1</sup> is i-Pr;

then R<sup>2</sup> is OMe, or OEt; R<sup>3</sup> is H; and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

e) when R<sup>3</sup> is Et;

then R<sup>2</sup> is H, R<sup>1</sup> is F or Cl, and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

f) when R<sup>2</sup> and R<sup>3</sup>, together with the phenyl carbons to which they are attached, form an ethylenedioxy ring;  
then R<sup>1</sup> is Me or Et and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

g) when R<sup>2</sup> and R<sup>3</sup>, together with the phenyl carbons to which they are attached, form a dihydrofuryl or dihydropyrryl ring;  
then R<sup>1</sup> is Et and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

h) when R<sup>1</sup> is formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OH, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>;  
then R<sup>2</sup> is OMe or OEt, R<sup>3</sup> is H, and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me; and

i) when R<sup>2</sup> is Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, O-n-Pr, OAc, NMe<sub>2</sub>, NET<sub>2</sub>, SMe, SET, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H, COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-i-Pr, SCN, SCHF<sub>2</sub>, SOMe, or NH-CN;  
then R<sup>1</sup> is Et, R<sup>3</sup> is H, the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

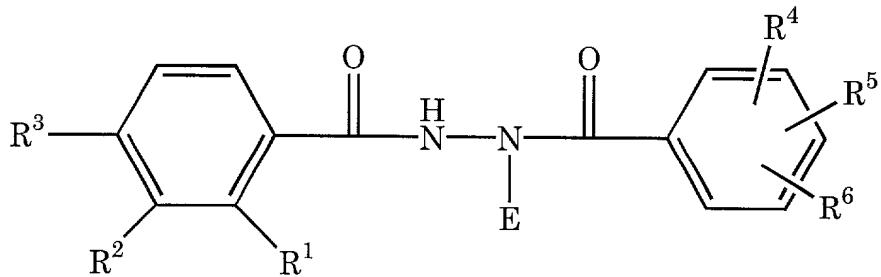
wherein the cells of the subject contain:

- a) an ecdysone receptor complex comprising:
  - 1) a DNA binding domain;
  - 2) a binding domain for the ligand; and
  - 3) a transactivation domain; and
- b) a DNA construct comprising:
  - 1) the exogenous gene; and
  - 2) a response element; and

wherein:

- a) the exogenous gene is under the control of the response element; and
- b) binding of the DNA binding domain to the response element in the presence of the ligand results in activation or suppression of the gene.

5. (Amended) A method for regulating endogenous or heterologous gene expression in a transgenic organism, wherein the organism is other than a plant, comprising contacting a ligand of the formula:



wherein:

E is a (C<sub>4</sub>-C<sub>6</sub>)alkyl containing a tertiary carbon or a cyano(C<sub>3</sub>-C<sub>5</sub>)alkyl containing a tertiary carbon;

R<sup>1</sup> is H, Me, Et, i-Pr, F, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>;

R<sup>2</sup> is H, Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe<sub>2</sub>, NET<sub>2</sub>, SMe, SET, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H, COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-i-Pr, SCN, SCHF<sub>2</sub>, SOMe, NH-CN, or joined with R<sup>3</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R<sup>3</sup> is H, Et, or joined with R<sup>2</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> are independently H, Me, Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SET;

provided that:

- a) when  $R^1$  is Me and  $R^2$  is OMe;  
then  $R^3$  is H; and the combination  $R^4$ ,  $R^5$ , and  $R^6$  is 3,5-di-Me, 3,5-di-OMe-4-Me, 3,5-di-Cl, or 3,5-di-F;
- b) when  $R^1$  is Me and  $R^2$  is OEt;  
then  $R^3$  is H and the combination  $R^4$ ,  $R^5$ , and  $R^6$  is 3,5-di-Me, 3,5-di-OMe-4-Me, 3,5-di-Cl, 3,5-di-F, 2,4- or 2,5-di-F, 2,4- or 2,5-di-Cl;
- c) when  $R^1$  is Et and  $R^2$  is OMe or OEt;  
then  $R^3$  is H and the combination  $R^4$ ,  $R^5$ , and  $R^6$  is:
  - i) 3,5-di-OMe-4-Me, 3,5-di-Cl, 3,5-di-F, 2,4- or 2,5-di-F, 2,4- or 2,5-di-Cl, 3-OMe, 2-Cl-5-Me, 2-Br-5-Me, 2-Cl, 2-Br, or 3-Me; or
  - ii)  $R^6$  is H,  $R^4$  is Me, and  $R^5$  is Et, F, Cl, Br, formyl,  $CF_3$ ,  $CHF_2$ ,  $CHCl_2$ ,  $CH_2F$ ,  $CH_2Cl$ ,  $CH_2OH$ , CN,  $C^{\circ}CH$ , 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SET;
- d) when  $R^1$  is i-Pr;  
then  $R^2$  is OMe, or OEt;  $R^3$  is H; and the combination  $R^4$ ,  $R^5$ , and  $R^6$  is 3,5-di-Me;
- e) when  $R^3$  is Et;  
then  $R^2$  is H,  $R^1$  is F or Cl, and the combination  $R^4$ ,  $R^5$ , and  $R^6$  is 3,5-di-Me;
- f) when  $R^2$  and  $R^3$ , together with the phenyl carbons to which they are attached, form an ethylenedioxy ring;  
then  $R^1$  is Me or Et and the combination  $R^4$ ,  $R^5$ , and  $R^6$  is 3,5-di-Me;
- g) when  $R^2$  and  $R^3$ , together with the phenyl carbons to which they are attached, form a dihydrofuryl or dihydropyryl ring;  
then  $R^1$  is Et and the combination  $R^4$ ,  $R^5$ , and  $R^6$  is 3,5-di-Me;
- h) when  $R^1$  is formyl,  $CF_3$ ,  $CHF_2$ ,  $CHCl_2$ ,  $CH_2F$ ,  $CH_2Cl$ ,  $CH_2OH$ ,  $CH_2OMe$ ,  $CH_2CN$ , CN,  $C^{\circ}CH$ , 1-propynyl, 2-propynyl, vinyl, OH, cyclopropyl,  $CF_2CF_3$ ,  $CH=CHCN$ , allyl, azido, SCN, or  $SCHF_2$ ;

then R<sup>2</sup> is OMe or OEt, R<sup>3</sup> is H, and the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me; and

- i) when R<sup>2</sup> is Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, O-n-Pr, OAc, NMe<sub>2</sub>, NEt<sub>2</sub>, SMe, SEt, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H, COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-i-Pr, SCN, SCHF<sub>2</sub>, SOMe, or NH-CN;

then R<sup>1</sup> is Et, R<sup>3</sup> is H, the combination R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> is 3,5-di-Me;

with an ecdysone receptor complex within the cells of the organism wherein the cells further contain a DNA binding sequence for the ecdysone receptor complex when in combination with the ligand and wherein formation of an ecdysone receptor complex-ligand-DNA binding sequence complex induces expression of the gene.

Add the following new claim:

20. (New) The method of Claim 3 wherein the subject is a fungus or yeast.

REMARKS

This application is a Divisional Application of Application Serial No. 09/315,451, filed May 20, 1999 which was a Continuation in Part of Application Serial No. 09/210,010, filed December 11, 1998, now abandoned..

Application Serial No. 09/315,451 was subject to restriction under 35 USC §121. This application is to prosecute the non-elected subject matter in Application Serial No. 09/315,451. New Claim 20 is supported by the Specification, page 9, line 30 to page 10, line 2.

Attached hereto is a marked-up version of the changes made to the Specification and claims by this preliminary amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Respectfully submitted,



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